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Binary monolayers of single-stranded oligonucleotides and blocking agent for hybridisation

Inger Vikholm-Lundin*, Reetta Piskonen[1]

VTT Technical Research Centre of Finland, P.O. Box 1300, FI-33101 Tampere, Finland

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ABSTRACT

The hybridisation of thiol-modified single-stranded DNA (SH-ssDNA) embedded in lipoate or hydrophilic polymer monolayers on gold have been studied through the technique of surface plasmon resonance (SPR). SH-ssDNA and the blocking agents were co-adsorbed on gold from the same solution or the surface was post-treated with the blocking agents. The assembling process is allowed to take place for only 10 min. Binding of non-complementary and complementary DNA is dependent both on the blocking agent and on the assembling method used. The lowest non-complementary binding and the highest complementary binding of DNA was obtained with a layer assembled from a binary solution of SH-ssDNA and the lipoate blocking agent.

1. Introduction

An efficient method for immobilising single-stranded nucleic acids (ssDNA) on a solid surface with preserved hybridisation specificity and reduced non-specific binding are of importance when improving the performance of DNA micro-arrays and developing DNA biosensors. Surfaces with ssDNA probes have been prepared by adsorption [1], copolymerization [2], complexation [3] and by covalent linkage [4–10]. Extensive studies have been performed on assembling thiol-tagged ssDNA probes on clean gold surfaces and post-treating the surface by mercaptohexanol (MCH) [4–6,9]. Mercaptohexanol has been used because it is water soluble, its carbon chain is of the same length as the spacer group of SH-ssDNA and it has been shown to resist non-specific binding ("NSB") of non-complementary target DNA. MCH is also thought to improve DNA probe orientation on the surface [4]. Surface hybridisation depends strongly on probe length and density, surface orientation and target sequence [4,8,11]. We have previously assembled SH-ssDNA on gold and post-treated the layer with a hydrophilic non-ionic polymer [12]. Alternatively, we assembled the components from the same solution. The polymer can be covalently attached onto the gold surface by disulphide anchors and has been used to reduce the "NSB" of biomolecules in immunosensors [13–15]. Thiol-tagged DNA and oligo(ethylene glycol) thiols have also recently been assembled from the same solution [16].

In this paper SH-ssDNA will be directly assembled onto a gold film and the remaining free space in between the oligos will be blocked by a lipoate, Lipa-DEA or a hydrophilic polymer, *N*-[tris(hydroxy-methyl)methyl]acrylamide, pTHMMAA. The lipoate and the polymer show low "NSB" and can be covalently attached onto the gold surface by disulphide anchors [13,17]. By using various oligo concentrations the surface coverage of the oligo-strands has been optimised. The assembling is allowed to take place for only 10–15 min either from the same solution or by post-treating the SH-ssDNA surface with the lipoate or the polymer. If DNA micro-arrays or biosensors are to be produced the assembling has to take place quite fast and an immobilisation procedure taking place overnight, the time normally used when assembling ssDNA on the surface cannot be used. The assembling procedure and the hybridisation were followed *in situ* by Surface Plasmon Resonance (SPR). The probe selected for this study can be used to detect napthalene dioxygenase (nahA) genes [18].

2. Materials and methods

2.1. Oligonucleoide sequences

Single-stranded, 15 bases long DNA (15-mer ssDNA) molecules thiol-modified in the 5′ end with the following sequence: HS-$(CH_2)_6$-CCC YGG CGA CTA TGT were used. The complementary and non-complementary DNA-strands were not thiol-modified and had

* Corresponding author. Tel.: +358 3 3163363; fax: +358 3 3163319.
E-mail address: inger.Vikholm-Lundin@vtt.fi (I. Vikholm-Lundin).
[1] Present address: WSP Environmental Oy, Heikkiläntie 7D, 00210 HKI, Finland.

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the sequence ACA TAG TCG CCR GGG (nahA-comp) and GGG RCC GCT GAT ACA (nahA-noncomp), respectively. The compounds were obtained HPLC purified from Sigma.

2.2. Lipoate derivative

The lipoate derivative Lipa-DEA was obtained by conjugation of lipoic acid (thioctic acid) and diethanolamine (DEA) [17] and the polymer, *N*-[tris(hydroxy-methyl)methyl]acrylamide, pTHMMAA was prepared as previously described (Scheme 1) [15]. A phosphate-buffered saline (PBS) pH 7.5 composed of 20 mM Na_2HPO_4/NaH_2PO_4, 300 mM NaCl, 1 mM EDTA pH 7.5 was used. EDTA and Na_2HPO_4 were purchased from Merck, NaCl and NaH_2PO_4 from J. T. Baker. Lipa-DEA was dissolved in ethanol and diluted in a PBS to a ratio of ethanol/PBS 1:10. The polymer, pTHMMAA was dissolved directly in PBS buffer.

2.3. Immobilisation procedure

Immobilisation of thiol-modified oligos and the lipoate, Lipa-DEA or the polymer, pTHMMAA and hybridisation experiments were carried out in a Biacore 3000 instrument (Biacore AB, Uppsala, Sweden). Glass slides coated with a 50 nm thin film of gold by sputter coating (using an Edwards E306A sputter coater) were cleaned in a hot hydrogenperoxide/ammoniumhydroxide/water solution (1/1/5) and rinsed with water. The slide was mounted in a plastic chip cassette by double-sides tape and inserted into the Biacore instrument. Because of this attachment of the slide the layer could not be regenerated in hot water, because rinsing with hot water removed the glass slide from the chip cassette.

The immobilisation procedure was started by rinsing the slide with buffer at a constant flow rate of 20 μl/min for 1 min. A binary solution of SH-ssDNA and Lipa-DEA or pTHMMAA was thereafter allowed to interact with the gold-coated surface typically for 10 min, followed by rinsing of the surface with the PBS buffer. Alternatively, SH-ssDNA was firstly allowed to flow over the surface. The surface was rinsed with buffer and secondly post-treated with the blocking agent, Lipa-DEA or pTHMMAA for the same time and rinsed with buffer.

2.4. Hybridisation procedure

The Biacore 3000 device was also used to determine the hybridisation of non-complementary and complementary strands *in situ* at room temperature. The oligonucleotides were dissolved in DNA-free water and diluted in PBS buffer. The oligonucleotiode sample was added at a flow rate of 20 μl/min for 10 min, after which the surface was flushed with buffer for 15 min. The target, nahA-comp was applied by titration when studying the hybridisation interaction.

3. Results and discussion

3.1. Assembling of SH-ssDNA and Lipa-DEA or pTHMMAA on gold

The inset in Fig. 1 shows that the assembly of the binary solution of SH-ssDNA and Lipa-DEA on Au surfaces is very fast. Saturation was obtained within 2 min. Thiols and disulfides form stable self-assembled monolayers (SAMs) on gold via a polar covalent bond. With thiols the reaction is assumed to take place as an oxidative addition to gold with release of hydrogen whereas in the case of disulfides, a cleavage of the S–S bond occurs. Disulfides, however, adsorb approximately 40% slower than thiols [19]. The kinetics is the same as when pure Lipa-DEA assembles on the surface (data not shown). The decrease in response in the dissociation phase is most probably due to a refractive index difference between buffer and

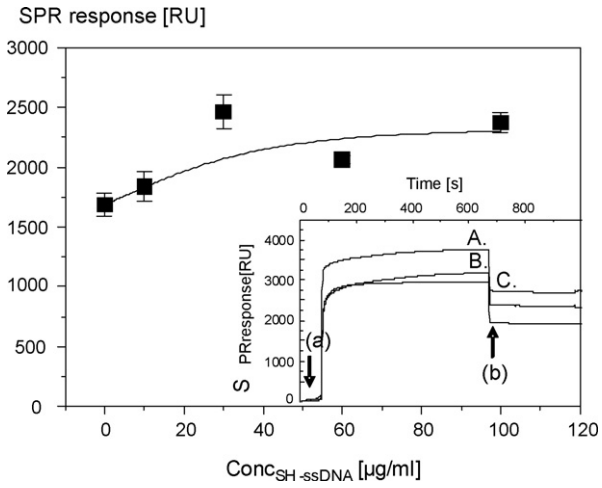

Fig. 1. Co-adsorption of increasing concentrations of SH-ssDNA and Lipa-DEA on gold. In the inset (a) denotes injection of binary solutions of (A) 100, (B) 30 and (C) 10 μg/ml SH-ssDNA and 0.2 g/l Lipa-DEA. Rinsing with buffer is denoted by (b). SH-oligos and Lipa-DEA were injected over a cleaned Au surface for 10 min and rinsed with buffer for 15 min.

the small amount of ethanol used to dissolve Lipa-DEA (2% ethanol). Lipa-DEA causes an SPR-response of 1650 ± 100 RU when injected over a clean gold surface at a concentration of 0.2 g/l. At this concentration a monolayer starts to form and at a concentration of 1 g/l the surface is saturated [17]. When SH-oligos were included into the mixing solution the response was higher than that of the pure Lipa-DEA solution. This response increased with SH-oligo concentration (Fig. 1 and Table 1). Saturation was observed above a concentration of 30 μg/ml with a response of 2300 ± 200 RU. If pTHMMAA was used as a blocking agent a response of 2970 ± 220 RU could be observed. This is expected as the polymer has a higher molecular weight.

When SH-ssDNA at a concentration of 60 μg/ml was adsorbed directly on gold the response was also 2300 ± 100 RU. An additional increase in response of 120 ± 20 and 290 ± 80 RU was obtained when post-treating the layers with Lipa-DEA and pTHMMAA, respectively (Table 1). A response of 1000 RU corresponds to a surface coverage of about 100 ng/cm^2 (http://www.biacore.com). This means that the surface coverage of the binary layers ranges from 230 to 300 ng/cm^2. The surface coverage is thus four- to five-fold to that reported for oligonucleotides immobilised on avidin (60 ng/cm^2), and slightly higher than that of covalently bond SH-ssDNA (40 pmol/cm^2) [20,21]. Thiol-modified ssDNAs are thought to adsorb on the surface through both the nitrogen-containing bases and the sulfur atom of the thiol group at low oligo concentration [4,22]. At low surface coverage much of the DNA lies flat on the surface probably with the DNA bases chemisorbed. Three cytosines are positioned close to the thiol group of the ssDNA sequence. The binding strength per mole of cytosine to gold is 128 kJ, whereas the thiol has a binding strength of 167 kJ/mole [23]. Because of the high surface coverage obtained at high SH-ssDNA concentrations the oligos are most probable anchored to the surface via the thiol groups and possibly via some of the cytosines. Lipa-DEA or the polymer, pTHMMAA is expected to be intercalated between the oligos.

3.2. Hybridisation of the layers

A considerable higher amount of non-complementary nahA was attached to the SH-oligo layer post-treated with pTHMMAA than when Lipa-DEA was used (Table 1). Very low amounts of non-complementary target were adsorbed when the layers were